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EXAMINER	
MONDESI, ROBERT B	

ART UNIT	PAPER NUMBER
1653	

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Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	09/976,673	LUKYANOV ET AL.	
	Examiner	Art Unit	
	Robert B. Mondesi	1653	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *12 October 2005*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-12 and 18-24* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-12 and 18-24* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *12 October 2001* is/are: a)☐ accepted or b)☒ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on October 12, 2005 has been entered.

Status of the claims

Claims 13-17 are canceled. **Claims 1-12 and 18-24** are presently pending and under examination.

Withdrawal of Objections and Rejections

Applicant's amendments and arguments filed have been fully considered and deemed to be persuasive to overcome some of the rejections and/or objections previously applied. Rejections and/or objections not explicitly restated from the previous Office action are hereby withdrawn.

Maintenance of rejections

The text of those sections of Title 35, U.S. Code not included in this action can be found in a prior Office action.

Double Patenting

Claims 1-2, 7-12 and 18-24 remain provisionally rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over **claims 1-5, 8-10, 12-15, 22-23** of copending Application No. 10006922.

Claims 1-2, 7-12 and 18-24 remain provisionally rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over **claims 1-3, 5-9 and 15-16** of copending Application No. 10081864.

Claims 1-12 and 18-24 remain provisionally rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over **claims 1-16, 21 and 43** of copending Application No. 10155809.

The above rejections were explained in the Office actions mailed June 1, 2005 and December 17, 2005.

Response to applicants' arguments

In response to the provisional rejection of **claims 1-2, 7-12 and 18-24** under the judicially created doctrine of obviousness-type double patenting over **claims 1-5, 8-10, 12-15 and 22-23** of copending Application No. 100006922; **claims 1-2, 7-12 and 18-24** over **claims 1-3, 5-9, and 15-16** of copending Application No. 10081864 and **claims 1-12 and 18-24** over **claims 1-16, 21 and 43** of copending Application No. 10155809, the applicants assert that claim 1 has been amended to recite "wherein said nucleic acid has a sequence similarity of at least about 75% with a nucleotide sequence of SEQ ID

NO:11", and in contrast the mentioned copending Applications above do not disclose the nucleic acid sequence of SEQ ID NO:11.

Applicants' arguments have not been found persuasive because even though the mentioned co-pending Applications above do not disclose SEQ ID NO: 11, they disclose nucleic acid fragments, wherein the nucleic acid fragment has a sequence similarity of at least about 75% with a nucleotide sequence of SEQ ID NO: 11. It is important to clarify and explain that the phrase " a nucleotide sequence" is not presently interpreted as " the nucleotide sequence"; therefore as a consequence, when the claim is interpreted according to its broadest reasonable interpretation, it encompasses fragments of any particular length that have a sequence similarity of at least about 75% with any segment/portion of the nucleotide sequence of SEQ ID NO: 11. Co-pending Applications No. 10006922, No. 10081864 and No. 10155809 disclose nucleic acid fragments that have a sequence similarity of at least about 75% with a nucleotide sequence of SEQ ID NO:11, for example nucleic acid designated as SEQ ID NO:39 of co-pending Application No. 100006922, nucleic acid designated as SEQ ID NO:21 of co-pending Application No. 10081864 and nucleic acid designated as SEQ ID NO:9 of co-pending Application No. 10155809, all contain fragments that are 100% identical to a segment/portion of SEQ ID NO:11 of the present application.

New Objection(s) and Rejection(s)

Abstract

The abstract is objected to because of the following informalities: the abstract discloses, "Nucleic acid compositions encoding *Stichodactylidaen* chromoproteins and

fluorescent mutants thereof, as well as the polypeptide compositions encoded by the same, are provided", as this appears to be redundant. The statement, "Nucleic acid compositions encoding *Stichodactylidaen* chromoproteins and fluorescent mutants thereof" is sufficient.

Drawings

The drawings are objected to because Figures 1-2, 4, 6, 8, 10, 12, 13, 15, 16 and 17 contain text that appear to be description of the drawing and not related to an Office acceptable legend under 37 CFR 1.84(o), such description belongs in the Brief Description of the Drawings in the specification of the application (The mentioned text appear above each Fig.). Corrected drawing sheets in compliance with 37 CFR 1.121(d) are required in reply to the Office action to avoid abandonment of the application. Any amended replacement-drawing sheet should include all of the figures appearing on the immediate prior version of the sheet, even if only one figure is being amended. The figure or figure number of an amended drawing should not be labeled as "amended." If a drawing figure is to be canceled, the appropriate figure must be removed from the replacement sheet, and where necessary, the remaining figures must be renumbered and appropriate changes made to the brief description of the several views of the drawings for consistency. Additional replacement sheets may be necessary to show the renumbering of the remaining figures. Each drawing sheet submitted after the filing date of an application must be labeled in the top margin as either "Replacement Sheet" or "New Sheet" pursuant to 37 CFR 1.121(d). If the changes are not accepted by the examiner, the applicant will be notified and informed of any required

corrective action in the next Office action. The objection to the drawings will not be held in abeyance.

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 1-2, 20-24 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter, which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

Claim 1 is drawn to a nucleic acid present in other than its natural environment, wherein said nucleic acid encodes a far red shifted *Stichodactylidaen* chromoprotein of fluorescent mutant thereof. Presently, **claim 1** has been amended to include the following limitation: "wherein said nucleic acid has a sequence similarity of at least about 75% with a nucleotide sequence of SEQ ID NO: 11". The specification on page 7, lines 25-27, discloses a variety of nucleic acids encoding mutant proteins, also on the same page (page 7), lines 29-35, it is asserted that, "in addition to the above described specific nucleic acid compositions, also of interest are homologues of the above sequences. With respect to homologues of the subject nucleic acids, the source

of homologous genes may be any species of plant or animal, or the homologue may be a completely synthetic sequence. In certain embodiments, sequence similarity between homologues is at least about 20%, sometimes at least about 25%, and may be 30%, 35%, 40%, 50%, 60%, 70% or higher, including 75%, 80%, 85%, 90% and 95% or higher". This type of Markush language disclosure is not considered to be sufficient written description support for the specific new limitation that attempts to provide a range of species for a genus of a nucleic acid molecule present in other than its natural environment, wherein said nucleic acid encodes a far red shifted *Stichodactylidaen* chromoprotein or fluorescent mutant thereof; therefore the limitation "wherein said nucleic acid has a sequence similarity of at least about 75% similarity with a nucleotide sequence of SEQ ID NO:11" is considered to be new matter. New or amended claims which introduce elements or limitations which are not supported by the as-filed disclosure violate the written description requirement. See, e.g., *In re Lukach*, 442 F.2d 967, 169 USPQ 795 (CCPA 1971) (subgenus range was not supported by generic disclosure and specific example within the subgenus range); *In re Smith*, 458 F.2d 1389, 1395, 173 USPQ 679, 683 (CCPA 1972) (a subgenus is not necessarily described by a genus encompassing it and a species upon which it reads). The specification of the present application does not disclose specific nucleic acid species that have a sequence similarity of at least about 75% with a nucleotide sequence of SEQ ID NO: 11, wherein the said species are encompassed by a genus of a nucleic acid that encodes a far red shifted *Stichodactylidaen* chromoprotein or fluorescent mutant thereof. **Claim 2** is a dependent claim that does not overcome the

deficiencies of the claim that it is depends therefrom. **Claims 20-24** are new claims that were added in amendment filed March 22, 2005 and did not appear in the application as originally filed on October 12, 2001; therefore these claims are also rejected for containing new matter. As mentioned above the specification does not provide a written disclosure for nucleic acid species having a sequence similarity of at least about 75% with a nucleotide sequence of SEQ ID NO: 11. As indicated previously above, the specification of the present application asserts that, "in certain embodiments, sequence similarity between homologues is at least about 20%, sometimes at least about 25%, and may be 30%, 35%, 40%, 50%, 60%, 70% or higher, including 75%, 80%, 85%, 90% and 95% or higher". Again, it is restated that, this type of Markush language disclosure cannot be considered to be sufficient written description support for the specific new limitation that attempts to provide a range of species for a genus of a nucleic acid. Accordingly, the specification fails to provide adequate written description support for the disclosure of nucleic acid species having a sequence similarity of at least about 75% with a nucleotide sequence of SEQ ID NO: 11.

Claims 1-2, 5-12 and 18-24 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for nucleic acid, SEQ ID NO: 11, encoding the mutant disclosed in example B.4, on page 33, lines 8-19, of the specification, Fp10-cr1 (hcFRFP-2) (HcRed-2A) does not reasonably provide enablement for nucleic acid wherein said nucleic acid encodes a far red shifted *Stichodactylidaen* chromoprotein or fluorescent mutant thereof wherein the said nucleic acid has a sequence similarity of at least about 75%, 80% or 90% with a nucleotide

sequence of SEQ ID NO: 11 or any fragment, mutant or mimetic thereof. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims.

The factors to be considered in determining whether undue experimentation is required are summarized In re Wands 858 F.2d 731, 8 USPQ2nd 1400 (Fed. Cir.1988). The court in Wands states: "Enablement is not precluded by the necessity for some experimentation such as routine screening. However, experimentation needed to practice the invention must not be undue experimentation. The key word is 'undue,' not 'experimentation.' " (Wands, 8 USPQ2d 1404). Clearly, enablement of a claimed invention cannot be predicated on the basis of quantity of experimentation required to make or use the invention. "Whether undue experimentation is needed is not a single, simple factual determination, but rather is a conclusion reached by weighing many factual considerations." (Wands, 8 USPQ2d 1404). The factors to be considered in determining whether undue experimentation is required include: (1) the breadth of the claims, (2) the nature of the invention, (3) the state of the prior art, (4) the relative skill of those in the art, (5) the predictability or unpredictability of the art, (6) the amount or direction or guidance presented, (7) the presence or absence of working examples, and (8) the quantity of experimentation necessary. Although the quantity of experimentation alone is not dispositive in a determination of whether the required experimentation is undue, this factor does play a central role. For example, a very limited quantity of experimentation may be undue in a fledgling art that is unpredictable where no

guidance or working examples are provided in the specification and prior art, whereas the same amount of experimentation may not be undue when viewed in light of some guidance or a working example or the experimentation required is in a predictable established art. Conversely, a large quantity of experimentation would require a correspondingly greater quantum of guidance, predictability and skill in the art to overcome classification as undue experimentation. In Wands, the determination that undue experimentation was not required to make the claimed invention was based primarily on the nature of the art, and the probability that the required experimentation would result in successfully obtaining the claimed invention. (Wands, 8 USPQ2d 1406). Thus, a combination of factors which, when viewed together, would provide an artisan of ordinary skill in the art with an expectation of successfully obtaining the claimed invention with additional experimentation would preclude the classification of that experimentation as undue. A combination of Wands factors, which provide a very low likelihood of successfully obtaining the claimed invention with additional experimentation, however, would render the additional experimentation undue.

1-2. Breadth of the claims and the nature of the invention.

The claims are overly broad in scope: **Claims 1** (**claim 2** dependent therefrom), **5** (**Claim 6** dependent therefrom), **7, 8, 9, 10 (claims 11-12** dependent therefrom) and **18-24** are so broad as to encompass a vast number of nucleic acid sequences wherein said nucleic acid sequence encodes a far red shifted *Stichodactylidaen* chrompoproetin of fluorescent mutant thereof wherein the said nucleic acid has a sequence similarity of at least about 75%, 80% or 90% with a nucleotide sequence of SEQ ID NO: 11. The

broad scope of claimed nucleic acid sequences is not commensurate with the enablement provided by the disclosure with regard to the mentioned nucleic acid molecule.

The claimed invention is also directed to a nucleic acid present in other than its natural environment that encodes a chromo or fluorescent protein or a mutant protein or a fragment of a nucleic acid or an isolated nucleic acid or mimetic thereof or a cell or a construct and expression cassette comprising same which include mutations that are not limited to a single point mutation and encompass a large variable genus.

3. The state of prior art.

The art provides evidence for the high degree of unpredictability in altering a protein sequence with an expectation that the protein will maintain the desired activity/utility. For example, Branden et al. ("introduction to Protein Structure", Garland Publishing Inc.,New York, 1991) teach "[p]rotein engineers frequently have been surprised by the range of effects caused by single mutations that they hoped would change only one specific and simple property in enzymes" and "[t]he often surprising results of such experiments reveal how little we know about the rules of protein stability. .. .. .they also serve to emphasize how difficult it is to design *de novo* stable proteins with specific functions" (page 247). While it is acknowledged that this reference was published in 1991, to date there remains no certain method for reasonably predicting the effects of even a single amino acid mutation on a protein.

Also of note, Heim et al. (PNAS, vol. 91 , pages 12501-04, 1994) disclose that a mutated DNA was sequenced and found to contain five amino acid substitutions, only

one of which was found to be critical, Tyr66His, in the center of the chromophore. Heim et al. also disclose further site directed mutagenesis and noted that there was tolerance of the substitutions made, however, some mutants were weakly fluorescent (page 12504). Therefore, amino acid substitutions are critical to the protein's structure/function relationship.

4. The relative skill in the art.

The relative skill in the art as it relates to the method of the invention is characterized by that of a M.D. or Ph. D. level individual.

5. The level of predictability in the art.

The high level of unpredictability in the art: The amino acid sequence of a protein determines the protein's structural and functional properties. Predictability of which changes can be tolerated in a protein's amino acid sequence and obtain the desired activity requires a knowledge of and guidance with regard to which amino acids in the protein's sequence, if any, are tolerant of modification and which are conserved (i.e., expectedly intolerant to modification), and detailed knowledge of the ways in which the protein's structure relates to its function. The positions within an encoding nucleic acid's sequence where modifications can be made with a reasonable expectation of success in obtaining an encoded polypeptide having the desired activity/utility are limited in any protein and the result of such modifications is highly unpredictable. In addition, one skilled in the art would expect any tolerance to modification for a given protein to diminish with each further and additional modification, e.g. multiple substitutions. In this

case, the necessary guidance has not been provided in the specification as explained above.

Furthermore, Rudinger (1976) Peptide Hormones, University Park Press, Baltimore, MD., pp. 1-7 discusses the peptide hormones and the characteristics of amino acids as components of the peptide hormones (TITLE). (It is noted that Rudinger discusses peptide hormones, but the general areas of unpredictability are common to all proteins.) In doing so, Rudinger notes that many amino acids may be grouped according to general characteristic (pp. 1-3), and many of these are also classified in two or more classifications (p. 3). Hence, simple mutations of "type" are not reasonably predictable, because there are multiple types to any particular amino acid. Moreover, Rudinger finds that the context of any amino acid is important for structure (pp. 3-4), and that therefore, simple deletions, insertions, or substitutions are also not reasonably predictable, because not only is "type" important, but context is also important, having longer-range effects than that of simply type. Further, Rudinger discusses the mechanisms of information transfer (e.g, binding and effecting a receptor, which is analogous to any protein binding anything and causing any particular effect) (pp. 4-5). In doing so, Rudinger finds that there exist "patterns" on molecules for recognition, which may involve amino acids close by in the amino-acid polypeptide sequence, or far away (Id.). As such the conformation of the whole molecule is important, and any particular amino acid change, deletion, or addition, may alter the conformation of the molecule enough to affect any particular binding and effect on another molecule.

In analyzing the significance of such observations, Rudinger states that:

> In a given molecule, some amino acids or sequences obviously owe their 'significance' to their inclusion in the pattern which is directly involved in recognition by, and binding to, the receptor. However, the fact that the existence of this pattern is dependent on a conformation stabilized by intramolecular interactions, ..., implies that other amino acids or sequences contributing to this conformational stability will be no less 'significant' for the biological activity of the molecule.

(p. 5).

And, in conclusion, Rudinger states:

> The significance of particular amino acids and sequences for different aspects of biological activity cannot be predicted *a priori* but must be determined from case to case by painstaking experimental study. The careful design of synthetic analogues, and their evaluation in biological systems which permit separate analysis of the various phases of hormone action, is the best way to obtaining such information.

(p. 6).

Bowie, et al. (1990) Science, 247 : 1306-10 provides similar insight into the lack of reasonable predictability for the mutation of any particular protein. To wit, Bowie discuses that while many substitutions may be tolerated, in other cases substitutions may not be tolerated at all (e.g., 1306, col. 2, paragraph 2). Moreover, the significance of surface and buried amino acids while is not reasonably predictable either (pp. 1306-07), surface sites may not have any importance, but sometimes they are absolutely important due to binding (p. 1308), and predicting structure with reasonable predictability is generally limited to homologous proteins, but even that is difficult due to alignment problems (p. 1308). In general, Bowie continues to reflect the observations of Rudinger: it is not reasonably predictable that any particular amino acid change, deletion, or addition would provide a functional molecule with similar activity, and only painstaking analysis would provide such information for any particular change (e.g., pp. 1309-10).

Hence, the nature of the invention is not reasonably predictable for any of the particular proteins and genes claimed, due to the unpredictability of structure-function relationships.

Thus, a skilled artisan would recognize the high degree of unpredictability, that the entire scope of nucleic acid sequences, including those encoding far red shifted fluorescent / chromphore proteins, would encode a polypeptide having the desired fluorescent or chromopheric activities.

6-7. The amount of guidance present and the existence of working examples.

The lack of guidance and working examples: The instant specification does not demonstrate or provide guidance as to what the structure of the protein will be once modified based on the changes contemplated in the claims and the instant specification (i.e. 75% sequence similarity or). No definition is provided for the phrase, which could encompass 75% or 80% or 85% etc., and said nucleic acid might not encode the same protein or be functional. In addition, the claims are directed to a complement or sequence that hybridize under stringent conditions, which cannot or may not encode the same protein. In the instant application, the properties of the protein recited in the claims (see for example claim 1) and the recitation of a nucleic acid encoding such is insufficient to determine a chemical structure for the mutants/fragments/mimetics encompassed in the claims. Additionally, there is no data provided demonstrative of a particular portion of the structure that must be conserved.

The specification provides 7 examples of *Stichodactylidaen* chromoprotein mutants on pages 32-34 of the specification; mutC148S (C143S, using self numbering), mut44-9

(A2S, T39A, C143S, L173H, P201L, 204E, using self numbering), mut44-6 (A2S, T36A, A65E, C143S, L173H, P201L, using self numbering), Fp10-cr1 (A2S, T36A, L122H, C143S, L173H, R168H, P201L, using self numbering), hc-4 (A2S, C143S, P201L, using self numbering), hc-44 (A2S, T36A, C143S, L173H, P201L, using self numbering) and hc-41 (A2S, T36A, L122H, C143S, L173H, P201L, using self numbering). The specification discloses that the mutants were generated using random mutagenesis of the primary fluorescent mutant mutC148S. A closer look at the mutants reveals that there are at least three and up to five mutations that are conserved among the produced mutants. All 6 mutants have the following conserved mutations: A2S, S143H and P201L (using self numbering) and five of the mutants (all except hc-4) have the following conserved mutations: A2S, T36A, S143H, L173H and P201L. Furthermore, of the produced mutants only mutC148 (primary mutant) mut44-9 and hc-44 have been disclosed to have a far red shifted emission spectra (Specification; page 34, line 9 and page 36, line 5). It is also of importance to note that the two mentioned mutants, mut44-9 and hc-44, contain five of the same conserved mutations indicated previously. The difference between mut44-9 and hc-44 is only the point mutation K204 E. Given that in actuality only a small and limited number of mutations have been disclosed in conjugation with the fact that only three mutants have been disclosed to have a far red shifted emission spectra, the specification fails provide adequate guidance that would allow a person skill in the art to establish a nexus between random mutagenesis of the wild type hcFP640 (*Stichodactylidaen* chromoprotein) and far red fluorescent emission activity.

Therefore, these working examples and guidance regarding additional signal mutations fail to provide the necessary guidance for making the entire scope of claimed nucleic acid sequences encoding far red shifted *Stichodactylidaen* chromoprotein having sequence similarity of at least about 75%, 80% or 90% with a nucleotide sequence of SEQ ID NO: 11.

8. The quantity of experimentation necessary.

The amount of experimentation that is required is undue: while methods of generating variants of a given protein, e.g., site-directed mutagenesis, are known, it is not routine in the art to screen for all proteins having a substantial number of modifications having any function, as encompassed by the instant claims. Therefore, in view of the overly broad scope of the claims, the lack of guidance and working examples provided in the specification, and the high degree of unpredictability as evidenced by the prior art, undue experimentation would be necessary for a skilled artisan to make and use the entire scope of the claimed invention.

Furthermore, it must be noted that the issue in this case is the breath of the claims in light of the predictability of the art as determined by the number of working examples, the skill level of the artisan and the guidance presented in the instant specification and the prior art of record. The Applicants make and test position is inconsistent with the decisions of *In re Fisher*, 427 F.2d 833, 839, 166 USPQ 18, 24 (CCPA 1970) where it is stated that "... scope of claims must bear a reasonable correlation to scope of enablement provided by the specification to persons of ordinary skill in the art...". Without sufficient guidance, determination of having the desired

biological characteristics is unpredictable and the experimentation left to those skilled in the art is unnecessarily and improperly extensive and undue. See *In re Wands*, 858 F.2d at 737, 8 USPQZd at 1404 (Fed. Cir. 1988). Therefore, for the instant specification to be enabling, it needs to provide direction/guidance regarding whether the structure of the chromo or fluorescent fragment/mutant can tolerate the modifications encompassed by claims and still possess the desired properties or whether a protein that does not have the desired properties may result. Absent sufficient guidance/direction one of skill in the art would not be able to practice the claimed invention commensurate in scope with the claims. Thus, for all these reasons, the specification is not considered to be enabling for one skilled in the art to make and use the claimed invention as the amount of experimentation required is undue, due to the broad scope of the claims, the lack of guidance and insufficient working examples provided in the specification and the high degree of unpredictability as evidenced by the state of the prior art, attempting to construct and test mutants of the claimed invention would constitute undue experimentation. Therefore, applicants have not provided sufficient guidance to enable one of skill in the art to make and use the claimed invention in a manner that reasonably correlates with the scope of the claims, to be considered enabling.

Claims 1-2, 5-12, 18-24 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

In **claims 1 and 5-6** applicants state that the nucleic acid molecule of the invention has a "sequence similarity" with a nucleotide sequence of SEQ ID NO: 11; however the applicants have not defined in what way the sequences are similar. The specification does not provide any particular definition that can be attributed to "sequence similarity"; therefore it is not clear as to what the applicants intend to cover by the recitation of the word similar (If the applicants intention is the use of the phrase "sequence identity", an amendment to the claim clarifying the claimed subject matter will overcome the rejection). **Claim 2** is a dependent claim that does not further clarify the deficiencies of the independent claim that it is dependent therefrom.

In **claims 7-10 and 18-24** the applicants use the phrase " having a sequence of similarity" in order to associate the nucleic acid of the invention with the nucleic acid sequence designated as SEQ ID NO: 11. It is not clear as to what the applicants intend to mean when using the phrase " having a sequence of similarity". Are the applicants indicating that the nucleic acid of the invention has sequence identity with an assortment of portions of SEQ ID NO: 11, the entire length of SEQ ID NO: 11 or fragments of SEQ ID NO: 11? It appears that the phrase has been constructed mistakenly and the actual intent is to state that, the nucleic acid molecule of the invention has sequence identity to the nucleic acid sequence of SEQ ID NO: 11; in which case an amendment to the claim correcting the mentioned phrase appropriately will overcome the rejection. **Claims 11-12** are dependent claims that do not further clarify the deficiencies of the independent claim, which they are dependent therefrom.

Claims 8 and 21 cite the phrase "mimetic thereof", whoever in sections a) and b) of the claims there is only reference to a nucleic acid and not a mimetic thereof. A nucleic acid encoding a protein can not be considered to be a "mimetic thereof", since it is not clear as to what the mentioned nucleic acid is a "mimetic thereof".

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (a) the invention was known or used by others in this country, or patented or described in a printed publication in this or a foreign country, before the invention thereof by the applicant for a patent.
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.
>
> (e) the invention was described in (1) an application for patent, published under section 122(b), by another filed in the United States before the invention by the applicant for patent or (2) a patent granted on an application for patent by another filed in the United States before the invention by the applicant for patent, except that an international application filed under the treaty defined in section 351(a) shall have the effects for purposes of this subsection of an application filed in the United States only if the international application designated the United States and was published under Article 21(2) of such treaty in the English language.

Claims 3-4, 7-12 and 18-24 are rejected under 35 U.S.C. 102(a) as being anticipated by Lukyanov et al., 2000.

The claims are drawn to a nucleic acid, a fragment of a nucleic acid, a nucleic acid molecule that hybridizes under stringent conditions to the said nucleic acid and a Kit, comprising a nucleic acid that encodes a fluorescent protein having an emission maximum ranging form about 620 to 680 nm. Also included is an expression cassette and a construct, comprising a vector and a nucleic acid molecule that encodes a fluorescent protein having an emission maximum ranging form about 620 to 680 nm and a method of expressing the said nucleic acid.

Lukyanov et al. teach that a homologue of GFP was found to determine the strong purple correlation of tentacles in the sea anemone *Anemonia sulcata* and under certain conditions; this novel chromoprotein produces a trace amount of red fluorescence (emission at 595nm). The quantum yield of the fluorescence can be enhanced dramatically by single amino acid replacement. Also it is mentioned that fluorescent variants of the protein have emission peaks that are red shifted up to 610nm (Page 25879, column 1, Abstract, lines 7-19).

Lukyanov et al. teach further that total RNA from the tips of the tentacles of *A. sulcata* was isolated. cDNA synthesis, amplification of the cDNA fragment of interest using degenerate primers, and obtaining the full length cDNA were performed. The full length coding region of asFP595 was cloned into pQE30 vector and the wild type protein as well as its mutant variants were expressed in *E. Coli* with 6xHis tag at the N-terminus and purified using TALON metal affinity resin. All preparations of the heterologous expression products were at least of 95% purity according to electrophoreses (Page 25879, column 2, lines 31-40) (**present claims 3-4, 7-12 and 18-24**)

Thus Lukyanov et al., 2000 teach all the elements of **claims 3-4, 7-12 and 18-24** and these claims are anticipated under 35 USC 102(a).

Claims 3-4, 7-12, 18-24 are rejected under 35 U.S.C. 102(b) as being anticipated by Min et al., 1999.

The claims are drawn to a nucleic acid, a fragment of a nucleic acid, a nucleic acid molecule that hybridizes under stringent conditions to the said nucleic acid and a Kit, comprising a nucleic acid that encodes a fluorescent protein having an emission maximum ranging form about 620 to 680 nm. Also included is an expression cassette and a construct, comprising a vector and a nucleic acid molecule that encodes a fluorescent protein having an emission maximum ranging form about 620 to 680 nm and a method of expressing the said nucleic acid.

Min et al. teach that it is generally accepted that two forms of (keto and enol) of oxyluciferin emit red and yellow-green light, respectively, and in order to demonstrate the existence of free emitting species using His-tagged luciferase, N-terminal 6x His-tagged luciferase was prepared and expressed in *E. Coli*. After immobilization of His-luc on the membrane, His-luc clearly showed spectral change toward red light. The luciferase-free product obtained from enzymatic reaction mixture in the presence of ATP and dATP emits the light with maximal wavelengths of 575 and 620 nm, respectively and based on the obtained results it is apparent that two different emitting species are responsible for two different color lights (Abstract, column 1, page 273, lines 1-16 and page 275, Figures 1.B and 3)

Min et al. teach further that for N-terminal 6X His tagged luciferase, the luc gene of pCMV-luc was amplificated using pFu polymerase and two primers and inserted into NheI/BamHI sites of plasmid pTrcHis (Invitorgen). His-luc was expressed in E. coli DH5alpha. Bacterial culture was in LB medium and it was induced by 1 mM IPTG (Column 1, page 274, lines 4-15) (**Present claims 3-4, 7-11, 18-24)**.

Min et al. also teach that the cell extracts were centrifuged and the supernatant was poured into a column and passed through and fractions with activity were pooled and concentrated (Page 274, column 1, lines 21-26 and 30-32)(**present claim 12**).

Thus Min et al., 1999 teach all the elements of **claims 3-4, 7-12, 18-24** and these claims are anticipated under 35 USC 102(b).

Claims 5-12, 18-24 are rejected under 35 U.S.C. 102(e) as being anticipated by Tsien et al. US Patent 6,342,379.

The claims are drawn to a nucleic acid having a sequence similarity or a sequence of similarity of at least about 75%, 80% or 90 % with a nucleotide sequence of SEQ ID NO: 11, a fragment of the mentioned nucleic acid, a nucleic acid that hybridizes under stringent conditions to the mentioned nucleic acid, a kit comprising the said nucleic acid, an expression cassette comprising the said nucleic acid, a construct comprising a vector comprising the said nucleic acid and a method of expressing the said nucleic acid.

It is important to clarify and explain that the phrase " a nucleotide sequence" is not presently interpreted as " the nucleotide sequence"; therefore as a consequence, when the claim is interpreted according to its broadest reasonable interpretation, it encompasses fragments of any particular length that have a sequence similarity of at least about 75% with any segment/portion of the nucleotide sequence of SEQ ID NO: 11.

Tsien et al. disclose a nucleic acid sequence that is 100% identical to a nucleotide sequence of at least 10 residues in length of SEQ ID NO: 11 (nucleotide sequence SEQ ID NO: 6 of US Patent 6,342,379, nucleotide 354 to 364) (**present claims 5-8, 18-21 and 24**).

Tsien et al. teach the construction of an expression vector utilizing the above nucleic acid sequence and a method of expressing the said expression vector (Column 34, lines 36-67 and column 35, lines 1-40) (**present claims 9-12 and 22-23**).

Thus, Tsien et al. teach all the elements of **claims 5-12 and 18-24** and these claims are anticipated under 35 USC 102(e).

Conclusion

No claims are allowed

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Robert B. Mondesi whose telephone number is 571-272-0956. The examiner can normally be reached on 9am-5pm, Monday-Friday.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Jon Weber can be reached on 571-272-0925. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

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